PAST ISSUE: **NOVEMBER - 2014** DARK MODE ISSN 0004 - **5772** VOLUME **62** | ISSUE : **ORIGINAL** : **ARTICLE**

Combining Fasting Plasma Glucose with Gamma-glutamyl Transferase Improves the Sensitivity to Predict Incident Diabetes in Asian Indian Men with Impaired Glucose Tolerance

Arun Nanditha[1], Ram Jagannathan[1], Selvam Sundaram[1], Priscilla Susairaj[1], Ananth Samith Shetty[1], Chamukuttan Snehalatha[1], Godsland F Ian[2], Desmond G Johnston[2], Ambady Ramachandran[1]

[1]India Diabetes Research Foundation and Dr. A. Ramachandran's Diabetes Hospitals, Chennai, India; [2]Faculty of Medicine, Imperial College, London

Received: 12.12.2013; Revised; 06.01.2014; Accepted: 06.01.2014;

Abstract

Objective: To study the associations of baseline gamma-glutamyltransferase (GGT) and alanine transaminase (ALT) with incident diabetes among Asian Indian men with impaired glucose tolerance (IGT).

Methods: In a 2 year prospective, randomised, controlled primary prevention study of diabetes, among 537 IGT men aged 35-55years, 123 incident diabetes (DM) cases occurred. Anthropometric {body mass index (BMI), waist circumference (WC)}, and laboratory measurements (fasting, 30 min and 2 hr plasma glucose (2 hr PG), HbA1c and plasma insulin, lipid profile, ALT, GGT) were estimated at baseline (Clinical Trial Identification No: NCT00819455). Predictive associations of baseline GGT and ALT values during the study were assessed using appropriate statistical methods.

Results: Baseline GGT but not ALT was significantly higher in incident diabetes cases. Mean (95%CI) GGT decreased in subjects who reverted to normal glucose tolerance (NGT), whereas it increased in subjects who deteriorated to diabetes (NGT:-3.5 (-6.4 to -0.6); IGT:-0.3 (-3.0 to 2.4); DM:8.3 (3.6 to 13.0) UL-1; P < 0.0001). The risk of DM significantly increased with increasing baseline GGT after adjusting for confounders such as BMI, alcohol drinking, 2 hr PG and insulin resistance (2.02[1.35-3.02]; P = 0.001). Receiver operating characteristic curve showed that the model comprising of baseline fasting plasma glucose (FPG) and GGT (area-under-curve(AUC)[95% CI]: 0.668[0.613-0.722]; P < 0.0001) was equally sensitive in identifying subjects with risk of diabetes as compared to 2 hr PG (AUC [95% CI]: 0.670[0.614-0.725]; P < 0.0001) and HbA1c (AUC[95% CI]: 0.677[0.619-0.734]; P < 0.0001) alone.

and sensitive tool to identify subjects at high risk of developing diabetes.

Clinical Trial No. NCT00819455

Introduction

The liver, a major site of insulin clearance, plays an important role in maintaining fasting and postprandial glucose homoeostasis. Unlike in the skeletal muscle, all triglycerides are stored intra-cellularly in the liver. Hence, hepatic fat accumulation affects insulin clearance, increases hepatic glucose output and the associated derangement of intermediary metabolism. It has been suggested that hepatic dysfunction resulting from the insulin resistance syndrome and non-alcoholic fatty liver disease (NAFLD) may contribute to the development of type 2 diabetes (T2DM).1 Evidence from several prospective studies in multi-ethnic populations provides a strong link between elevated liver enzymes such as gamma-glutamyl transferase (GGT)2-5 and alanine transaminase (ALT)2,4,5 and development of diabetes.

There are, however, several shortcomings in the current evidence base: firstly, incident diabetes has been generally determined from self-reported information in medical records or the diagnosis was based on single fasting glucose measurements.4 This could lead to potential misclassification bias. Furthermore, except two studies2,4 other studies did not adjust for confounding variables such as insulin resistance, 2 hr PG and HbA1c. Hence, we have studied the association of hepatic enzymes (GGT, ALT) with incident diabetes, ascertained in a prospective epidemiological setting, after adjusting for a comprehensive array of covariates known to be associated with diabetes risk.

Material and Methods

The study group consisted of 537 Asian Indian men with impaired glucose tolerance (IGT) who participated in a 2 year prospective diabetes prevention programme in India. The complete study design, eligibility criteria, recruitment of participants, and methods have been described elsewhere.6 Briefly, the eligible IGT subjects were randomly assigned

to one of the two treatment groups: the control group (n = 266) which received standard advice on healthy lifestyle practice at baseline. The intervention group (n = 271) received a constant reminder about healthy lifestyle principles through automated, tailored, mobile phone based text messaging system for two years in addition to the baseline standard care advice. The study showed for the first time that motivation through SMS could help to reduce the incidence of diabetes in high risk subjects. The study protocol was approved by the Ethical Review Committee of the India Diabetes Research Foundation (IDRF), Chennai, India. The study participants gave written informed consent before enrolment into the study.

All the participants were followed-up two years at 6 monthly intervals to ascertain the progression to diabetes. Diabetes was diagnosed on the basis of World Health Organisation (WHO)7 criteria using the oral glucose tolerance test (OGTT)- a plasma glucose of 126 mg /dl (7.0 mmol/l) or higher in the fasting state and /or 200 mg /dl or higher two hours after a 75-g oral glucose load. The OGTT with 3 blood sampling (fasting, 30 and 120 minutes) were carried out at annual visits. During the interim visits (6 and 18 months), only a 2 hr post glucose load test was carried out to minimise patient inconvenience. If the capillary blood glucose value was 200 mg/dl or greater, a 2 hr OGTT was done within 1 week.

Anthropometric, haemodynamic and biochemical measures were estimated as reported previously.6 All the biochemical assays were performed on a Cobas Integra 450 plus (Roche Diagnostics, Germany) autoanalyser using reagents from Roche diagnostics, with appropriate quality control (Roche diagnostics reference serum). Plasma insulin was estimated using an electrochemiluminescence assay in an elesysCobas e411 auto-analyser (Roche diagnostics, Germany). Participant's dietary energy intake and physical activity assessments were made using validated methods used in our previous prevention programmes.6 Of the 537 subjects recruited, 517 responded to the final follow-up (response rate: 96.3%). GGT values were available for 505 of the 517 who completed the 2nd year follow-up and they were included in the analysis (data from intervention and control groups combined).

Insulin resistance was calculated as HOMA-IR (fasting glucose (mg/dl) X insulin (mU/l)/ 405).8 The insulinogenic

(Î”I0-30/ G30).9

Statistical Analysis

Descriptive statistics of baseline measurements were computed by the median values of baseline GGT levels. GGT levels at baseline and at the end of the study stratified based on glycaemic status was analysed by one way ANOVA with Dunnetposthoc correction. Within group differences in GGT levels stratified based on glycaemic categories was assessed using paired t-test. Cox's proportional hazard models were computed to assess the re ve risk of GGT with incident diabetes after adjusting for potential confounding variables. Analyses were adjusted for dichotomous variables: family history, smoking and drinking habits and continuous variables: baseline age, body mass index (BMI), waist circumference (WC), 2 hr PG, HbA1c, Triglycerides(TG), ALT and HOMA-IR. In order to determine the predictive power of baseline GGT and its additive effect over FPG and 2 hr PG in predicting diabetes, a non-parametric, receiver operating characteristic (ROC) analyses were performed with the cumulative incidence of diabetes at the end of 2 years as the outcome variable.

Results

During the 2 year follow up, there were 123 incident cases of diabetes among 505 non-diabetic men in this cohort. Mean age and BMI were 46.0 ± 4.7 years and 25.8 ± 3.1 kg/m2 respectively. There was no difference in the mean age (DM: 46.1 ± 4.6 vs. Non-DM: 46.1 ± 4.8) and BMI between converters (diabetes) and non-converters (DM: 25.8 ± 3.2 vs. Non-DM: 25.9 ± 3.2). The median values of serum GGT and ALT were 24.0 (inter quartile range (IQR: 18.0 - 36.1)) UL-1 and 13.0 (IQR: 10 - 19) UL-1 respectively. Alcohol was consumed regularly by 38.0% of the study subjects and 23.6% were current smokers. The levels of GGT were significantly higher in those who developed diabetes at the end of the study (IGTâ†’ NGT: 23.0 (17.9 - 33.8) UL-1; IGT â†’ IGT: 23.0 (17.4 - 35.5) UL-1; IGT â†’ DM: 30.1 (22.6 - 48.8) UL-1; P for trend < 0.0001). The levels of ALT did not differ statistically between the groups (NGT: 14.7 ± 9.9 UL-1; IGT: 14.6 ± 7.2 UL-1; DM: 16.5 ± 9.4 UL-1, P for trend = 0.101). Hence, for the subsequent analysis, we studied only the effect of GGT on the incidence of diabetes.

Table 1 : Characteristics of the study participants based on their baseline GGT (values in medians)

Variables	Group-1 (GGT < 24.0 IU/L) n= 257	Group-2 (GGT ≥ 24.0 IU/L) n =248	P Value
	(Mean ± SD)		
Age (Yrs)	46.2 ± 4.7	45.9 ± 4.6	0.501
BMI (Kg/m^2)	25.5 ± 3.1	26.2 ± 3.2	0.013
Waist circumference (cm)	92.0 ± 7.2	93.2 ± 7.5	0.061
Family history of diabetes n (%)	141 (54.9)	130 (52.4)	0.582
Smoking n (%)	52 (20.2)	63 (25.4)	0.170
Drinking n (%)	78 (30.4)	109 (44.0)	0.002
Blood Pressure (mmHg)			
Systolic	123.7 ± 14.8	122.6 ± 12.7	0.376
Diastolic	79.6 ± 8.6	80.8 ± 8.2	0.134
Plasma Glucose (mg/dl)			
Fasting	100.9 ± 9.2	101.7 ± 10.1	0.356
2hr	156.6 ± 14.0	159.4 ± 15.3	0.030
HbA1c % (mmol/mol)	6.1 ± 0.3 (43.2 ± 3.3)	6.2 ± 0.4 (44.3 ± 4.4)	0.001
Alanine transaminase (IU/L)	13.8 ± 8.8	16.2 ± 7.8	0.002
Lipid profile (mg/dl)			
Triglycerides*	125.0 (95.0 - 166.5)	161.0 (118.0 - 218.3)	< 0.0001
Cholesterol	183.2 ± 33.3	193.1 ± 36.7	0.002
High Density Lipoprotein-Cholesterol	34.9 ± 7.7	34.3 ± 7.9	0.370
HOMA-IR	2.9 ± 1.5	3.3 ± 1.3	0.007
Insulinogenic			

continuous variables and counts (percentage) for categorical variables. Data analysed using two-sided independent sample t-test or Mann-Whitney U test. $P < 0.05$ considered significant

Distributions of anthropomorphic, haemodynamic, and metabolic variables in the two categories of GGT levels are presented in Table 1. The subjects with higher than the median value of GGT (group 2) had raised BMI, higher rates of alcohol consumption, 2 hr PG, HbA1c, abnormal lipid profile and had increased insulin resista e. We observed significant univariate relationships of baseline GGT with many of these variables. The strongest associations with baseline GGT were with TG, Total cholesterol (TC), ALT, HbA1c and HOMA-IR ($P < 0.0001$). Current alcohol drinking habits and obesity measures (BMI and WC) were also correlated with baseline GGT levels ($P < 0.01$). Haemodynamic measures and high density lipoprotein (HDL-C) did not correlate significantly with GGT levels.

Table 2 : Cox proportional hazard model showing the predictive power of baseline GGT

Variables	β (SE)	HR [95% CI]	P value
Model - 1			
GGT (≥ median vs < median)	0.79 (0.20)	2.18 [1.48-3.21]	<0.0001
Model - 2			
GGT (≥ median vs < median)	0.57 (0.21)	1.78 [1.17-2.68]	0.007

Dependent variable: incident diabetes vs non-diabetes; GGT values are dichotomised into below median (< 24 UL^{-1}) and above median (> 24 UL^{-1}) values; Model - 1: GGT adjusted for age, BMI, family history of diabetes, smoking, drinking ALT levels; Model - 2: model - 1 further adjusted for baseline 2 hr OGTT plasma glucose, HbA1c, triglycerides, HOMA-IR (measure of insulin resistance) and insulinogenic index (measure of beta cell function).

In the total group, over the two years, the median GGT level did not change significantly (final: 25.0 (19.0 - 37.0)

(95%CI) GGT decreased in subjects who reverted to normal glucose tolerance (NGT), whereas it increased in subjects who developed diabetes (NGT: - 3.5 (- 6.4 to - 0.6); IGT: - 0.3 (- 3.0 to 2.4); DM: 8.3 (3.6 to 13.0) UL-1; P < 0.0001). There was no significant change in GGT levels in subjects who remained as IGT.

Cox's proportional hazard model showed that higher concentrations of GGT were significantly associated with increased risk of developing diabetes after adjustment for potential confounders known to affect circulating GGT, such as alcohol drinking and insulin resistance (Table 2). In model - 2, higher levels of GGT (≥ median) showed a hazard ratio of 1.78 [95%CI: 1.17 - 2.68]; P = 0.007) when compared with the lower values. The effect of GGT was independent of the levels of 2 hr PG, TG, insulin resistance (HOMA-IR) and insulinogenic index.

ROC analyses showed that the baseline GGT levels of ≥ 26.4 UL-1 predicted incident diabetes over a 2 year period (AUC [95% CI]: 0.637 [0.581 - 0.692]; P < 0.0001; sensitivity: 61.2%; specificity: 60.9%). As expected baseline FPG ≥ 101 mg/dl (AUC [95%CI] : 0.615 [0.558 - 0.672]; P < 0.0001), 2hr PG ≥ 155 mg/dl (AUC [95%CI]: 0.670 [0.614 - 0.725]; P < 0.0001) and HbA1c ≥ 6.1%(AUC [95%CI]: 0.674 [0.616 - 0.732]; P < 0.0001) predicted incident diabetes in this cohort. Among the glycaemic measures, baseline levels of HbA1c showed the highest predictive value for detecting diabetes. In separate analyses, we have studied the predictive utility of GGT when combined with the glycaemic measures (Table 3). The results showed that the model comprising of FPG and GGT (AUC [95% CI]: 0.668 [0.613 - 0.722]; P < 0.0001) was equally effective in identifying subjects with risk of diabetes as compared to the 2 hr PG or HbA1c. Furthermore, the addition of GGT to glycaemic measures improved the sensitivity for detecting diabetes.

Table 3 : ROC analyses showing the specificity and sensitivity of the additive effect of GGT in predicting incident diabetes compared to FPG and 2 hr PG alone

Variables	AUC (95% CI)	Specificity (%)	Sensitivity (%)
GGT	0.637	61.2	60.9

(mg/dl)	(0.558 - 0.672)		
Fasting plasma glucose (mg/dl)+ GGT (median)	0.668 (0.613 - 0.722)	62.8	60.7
2 hr plasma glucose (mg/dl)	0.670 (0.614 - 0.725)	63.6	57.8
2 hr plasma glucose (mg/dl) +GGT (median)	0.705 (0.653 - 0.757)	66.9	64.1
HbA1c %	0.677 (0.619 - 0.734)	67.5	60.2
HbA1c + GGT	0.702 (0.648 - 0.755)	69.4	57.3

Discussion

Elevated GGT levels in prediabetic subjects were associated with increased risk of developing diabetes even after adjustment for potential confounding variables. GGT levels above the median range of 24.0 UL-1 were predictive of incident diabetes. The present threshold of ≥ 26.4 UL-1 in the ROC analysis was much lower than that of conventional upper limit of normal (50 UL-1) for GGT. Revision of the current normal concentration range may be warranted if GGT is to be used in prediction of diabetes.10 Previous large, epidemiological, prospective studies demonstrated an association between elevated GGT levels and risk of diabetes in normoglycaemic subjects among white,2-4 Black,11 Japanese12 and Korean populations.13 The present analysis provides new evidence about the link between GGT and incident diabetes in Asian Indian subjects with IGT.

In this cohort of subjects with IGT, GGT levels decreased in those subjects who regressed to NGT, whereas it increased in those who deteriorated to diabetes. An increase in GGT over 2 years was predictive of incident diabetes irrespective of baseline GGT levels. This finding was in accordance with the previous prospective studies which showed a positive association of changes in GGT with T2DM,3 metabolic syndrome14 and cardiovascular disease.15

Increased GGT is conventionally interpreted as a marker of excessive alcohol consumption. This relationship did not

be due to the excessive lipid accumulation in hepatocytes and the resultant hepatic insulin resistance possibly related to decreased portal insulin extraction and increased glucose output, thereby contributing to the development of total body insulin resistance and diabetes.16 However, in this study the association of GGT with incident diabetes was independent of insulin resistance as measured by HOMA-IR. Therefore, the association of GGT with diabetes could be through other alternate pathways other than hepatic insulin resistance. One possible explanation is that, even mild chronic dysglycaemia (as in IGT) is a pathological condition which causes damage to intracellular systems through oxidative stress. Raised GGT levels might be a result of oxidative stress17 which might play a role in causation and development of diabetes.

No independent association between raised ALT levels and incident diabetes was seen in our population. This finding was in accordance with the findings from some18,19 but not all2,5 previous studies.

The existing glucose based diagnostic tests have a few performance limitations.20 The OGTT is the gold standard test for the diagnosis of diabetes according to WHO. But it is time-consuming, unpleasant to the patients and not used routinely in large epidemiological studies. Though, FPG is easy to perform it has poor specificity for identifying high risk subjects, especially in Asian populations. Recently, American Diabetes Association and the WHO21 recommended HbA1c for diagnosis for diabetes. But, HbA1c also has some few key limitations as a screening tool due to multiple methodological differences and interferences including ethnic variations.22 Hence, there is a need to identify inexpensive, routine and standardised methods to diagnose and screen high risk subjects for diabetes. In our cohort, the combination of FPG and GGT (specificity: 62.8%; sensitivity: 60.7%) was found to be better in identifying subjects at high risk for diabetes compared with FPG alone (specificity: 62.8%; sensitivity: 53.6%). In fact, the combination was as predictive as the 2 hr PG and HbA1c for this purpose. In an epidemiological setting, the use of OGTT and HbA1c may be limited. Therefore, we propose that a simple clinical model comprising of FPG and GGT which are routinely measured, as an alternate screening tool to identify subjects with high risk of developing diabetes. These findings could have important public health implications.

Arun Nanditha : Researched, Analysed, Discussed and Edited Manuscript; Jagannathan Ram : Researched, Analysed, Discussed and Edited Manuscript; Sundaram Selvam : Contributed to discussion, Reviewed manuscript ; Susairaj Priscilla : Contributed to discussion, Reviewed manuscript; Ananth Samith Shetty : Contributed to discussion, Reviewed manuscript; Chamukuttan Snehalatha : Researched, Analysed, Discussed and Edited Manuscript; Ian F Godsland : Contributed to discussion, Reviewed manuscript; Desmond G Johnston : Contributed to discussion, Reviewed manuscript; Ambady Ramachandran : Researched, Analysed, Discussed and Edited Manuscript

Acknowledgements

We acknowledge the service by our epidemiology team consisting of Mary Simon, C.K. Sathish Kumar, K. Tamil Selvan. We thank R. Sekar for the help rendered in laboratory estimations. We also thank L. Vijaya for helping in statistical analysis and secretarial assistance.

Funding

The main study was funded by the UK India Education and Research Initiative (UKIERI, IND/CONT/06-07/187E). We also acknowledge partial funding from the World Diabetes Foundation (WDF) (WDF 08 406).

Conflict of Interest

There are no conflicts of interest.

Abbreviation

ALT : Alanine Transaminase; AUC : Area-Under-Curve; BMI : Body Mass Index; DM : Diabetes; ELICA : Electrochemiluminescence Assay; FPG : Fasting Plasma Glucose; GGT : Gammaglutamyl Transferase; HDL-C : High Density Lipoprotein Cholesterol; IDRF : India Diabetes Research Foundation; IGI : Insulinogenic Index; IGT : Impaired Glucose Tolerance; NAFLD : Non-Alcoholic Fatty Liver Disease; NGT : Normal Glucose Tolerance; OGTT : Oral

TG : Triglycerides; WC : Waist Circumference; WHO : World Health Organisation.

References

1. Clark JM, Diehl AM: Nonalcoholic fatty liver disease: an underrecognized cause of cryptogenic cirrhosis. JAMA 2003;289:3000-04.
2. Fraser A, Harris R, Sattar N, Ebrahim S, Davey Smith G, Lawlor DA: Alanine aminotransferase, gamma-glutamyltransferase, and incident diabetes: the British Women's Heart and Health Study and meta-analysis. Diabetes Care 2009;32:741-50.
3. Andre P, Balkau B, Born C, Charles MA, Eschwege E: Three-year increase of gamma-glutamyltransferase level and development of type 2 diabetes in middle-aged men and women: the D.E.S.I.R. cohort. Diabetologia 2006;49:2599-603.
4. Wannamethee SG, Shaper AG, Lennon L, Whincup PH: Hepatic enzymes, the metabolic syndrome, and the risk of type 2 diabetes in older men. Diabetes Care 2005;28:2913-18.
5. Sattar N, Scherbakova O, Ford I, O'Reilly DS, Stanley A, et al: Elevated alanine aminotransferase predicts new-onset type 2 diabetes independently of classical risk factors, metabolic syndrome, and C-reactive protein in the west of Scotland coronary prevention study. Diabetes 2004;53:2855-60.
6. Ramachandran A, Snehalatha C, Ram J, Selvam S, Simon M, Nanditha A, et al: Effectiveness of mobile phone messaging in prevention of type 2 diabetes by lifestyle modification in men in India: a prospective, parallel-group, randomised controlled trial. Lancet Diabetes Endocrinol 2013;1:191-8.
7. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 23 Suppl 1:S4-19, 2000.
8. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-19.

glucose tolerance test as a measure of insulin secretion. Diabet Med 1995; 12:931.

10. Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, et al: Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med 2002;137:1-10.

11. Schneider AL, Lazo M, Ndumele CE, Pankow JS, Coresh J, Clark JM, et al: Liver enzymes, race, gender and diabetes risk: the Atherosclerosis Risk in Communities (ARIC) Study. Diabet Med, 2013;30:926-33.

12. Doi Y, Kubo M, Yonemoto K, Ninomiya T, Iwase M, Tanizaki Y, et al: Liver enzymes as a predictor for incident diabetes in a Japanese population: the Hisayama study. Obesity (Silver Spring) 2 7;15:1841-50.

13. Kim CH, Park JY, Lee KU, Kim JH, Kim HK: Fatty liver is an independent risk factor for the development of Type 2 diabetes in Korean adults. Diabet Med 2008;25:476-81.

14. Ryu S, Chang Y, Woo HY, Yoo SH, Choi NK, Lee WY, et al: Longitudinal increase in gamma-glutamyltransferase within the reference interval predicts metabolic syndrome in middle-aged Korean men. Metabolism 2010;59:683-89.

15. Emiroglu MY, Esen OB, Bulut M, Karapinar H, Kaya Z, Akcakoyun M, et al: GGT levels in type II diabetic patients with acute coronary syndrome (does diabetes have any effect on GGT levels in acute coronary syndrome?). ActaDiabetol 2013;50:21-25.

16. Gronbaek H, Thomsen KL, Rungby J, Schmitz O, Vilstrup H: Role of nonalcoholic fatty liver disease in the development of insulin resistance and diabetes. Expert Rev GastroenterolHepatol 2008;2:705-11.

17. Lee DH, Blomhoff R, Jacobs DR, Jr.: Is serum gamma glutamyltransferase a marker of oxidative stress? Free Radic Res 2004;38:535-39.

18. Nannipieri M, Gonzales C, Baldi S, Posadas R, Williams K, Haffner SM, et al: Liver enzymes, the metabolic syndrome, and incident diabetes: the Mexico City diabetes study. Diabetes Care 2005;28:1757-62.

19. Schindhelm RK, Dekker JM, Nijpels G, Heine RJ, Diamant M: No independent association of alanine aminotransferase with risk of future type 2 diabetes in the Hoorn study. Diabetes Care 2005;28:12.

20. GA Nichols THaJB: Progression from newly acquired impaired fasting glucose to type 2 diabetes. Diabetes

Subscribe JAPI

0
SHARES